Biochemical Studies on the Effect of Volatile Oil of *Thymus capitatus* in Alloxan-Induced Diabetic Rats

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**Abstract**

Administration of thyme oil @ 0.1 mL kg⁻¹ body weight orally once daily for 28 days to diabetic rats revealed progressively declined values of serum glucose from 483.30±1.35 mg% on day 0-113.20±1.00 mg% at the end of the trial. Serum total cholesterol levels revealed progressively inclining values till 21 days and thereafter declining to 107.40±1.20 mg% at the end of the trial. However, serum HDL, triglycerides, serum urea nitrogen and creatinine levels remained within normal limits as recorded in glibenclamide treated diabetic rats except a mild increase in ALT and AST levels was recorded on day 7. The histological findings revealed regenerative changes in the pancreas, liver and kidney. The present results demonstrated the antihyperglycemic and antilipidemic effects of thyme oil.

**Key words**: Antihyperglycemic, thyme oil, biochemical changes, alloxan induced diabetic rats

Diabetes mellitus, one of the most common metabolic disorders affecting nearly 10% of world population has a significant impact on health, quality of life and life expectancy of patients as well as on the health care system. Its incidence is increasing rapidly at global level, particularly in developing countries. Despite the presence of known antidiabetic medicines in the pharmaceutical market, diabetes and the related complications continue to be a major health problem. On the other hand, traditional medicinal plants have been used since the ancient times by physicians and laymen to treat diabetes and its related complications, presenting a stirring prospect for the expansion of an alternative way of treatment of this disease. Herbal drugs are prescribed widely, even when their biologically active compounds are unknown, because of their effectiveness, lesser side-effects and relatively low cost. There are more than 1200 species of medicinal plants recognized throughout the world for their ability to treat diabetes mellitus. To date only a few of the medicinal plants grown in Africa have been used in folk medicine the treatment of diabetes mellitus (22,35). The whole plant as such or its various parts namely seeds, fruits, flowers, leaves, bark, stem, roots, bulbs, sprouts, corms and immature pods have been commonly employed in folk medicine to treat diabetes mellitus.

Of various plants grown in Libya, *Thymus capitatus*, a perennial aromatic sub-shrub native to the western Mediterranean area has been proven widely to possess various medicinal
properties including antimicrobial, antifungal, antioxidant, antitumorogenic, antimutagenic and anti-inflammatory activities (9,20,25). However, no studies have been reported on the antidiabetic effect of this plant.

Therefore the present study was carried out to assess the antidiabetic and related effects of volatile oil extracted from T. capitatus in alloxan-induced diabetic rats.

**Materials and Methods**

**Plant material and extraction of volatile oil:**
The fresh leaves of T. capitatus were collected from the areas of south of El Beida to Laruloda, Libya. The authenticity of the plant species was identified by scientists in the Department of Botany, Faculty of Science, Al Fateh University, Tripoli, Libya. The volatile oil was extracted by hydrodistillation method as per the procedure described by Balbaa et al. (8).

**Chemicals and drugs:** Alloxan monohydrate was purchased from Sigma Chemicals (St. Louis, USA). All the biochemical kits used in this experiment were obtained from Bicon diagnostik, Germany and all the other chemicals used were of analytical grade.

**Animals:** Male albino rats weighing 100-150 g (bred in the Animal House, Omar Al Mukhtar University, Al Beida, Libya) were used in the present experiment. The animals were housed in polypropylene cages, fed on a standard pellet diet and water given *ad libitum*. All the studies were conducted in accordance with the NRC (24).

**Experimental induction of diabetes:** Animals were deprived of feed for 24 h but were allowed free access to water before administration of alloxan. Alloxan monohydrate was dissolved in sterile normal saline and administered @ 150 mg kg⁻¹ body weight intraperitoneally as a single dose (6). The rats found hyperglycemic after 48 h of alloxan administration, with blood glucose levels above 250 mg dL⁻¹ were used for further studies.

**Design of the experiment:** A total number of 60 rats were used and they were divided into 6 groups of 10 rats each. Non-diabetic rats were used for the group I and II while diabetic rats were used for the remaining groups. Group I served as non-diabetic control which received no treatment while group II, consisted of non-diabetic rats, received volatile oil of thyme @ 0.1 mL kg⁻¹ body weight. Group III consisted of diabetic rats which received corn oil @ 0.1 mL kg⁻¹ body weight and group IV consisted of diabetic rats treated with volatile oil of thyme @ 0.1 mL kg⁻¹ body weight. Group V served as drug control (diabetic rats treated with glibenclamide @ 5 mg kg⁻¹ body weight) while group VI served as diabetic control which received no treatment.

**Administration of volatile oil and Glibenclamide:** The volatile oil of thyme was dissolved in corn oil while glibenclamide was dissolved in distilled water and administered orally using a feeding needle. All doses were administered orally once daily for a period of 28 days.

**Collection of blood samples:** Blood samples were collected at 0, 7, 14, 21 and 28 days of the trial from the orbital sinus using capillary tubes after partly anaesthetizing the rats.

**Parameters studied**

**Biochemical estimations:** Serum glucose was determined by GOD-POD method (32), total cholesterol was determined by CHOD-PAP method (3) and triglycerides concentration was determined by GPO-PAP method (15). Serum HDL and LDL concentrations were determined by the method described by Rifai and Warnick (27). Serum urea nitrogen was determined by diacetyl monoxine method (33) and creatinine was determined by alkaline picrate method (31). Alanine amino transferase (ALT)
and Aspartate amino Transferase (AST) activities in the serum were determined by Reitman and Frankel (26) method.

Pathological studies: One rat from each group was sacrificed at 0, 7, 14, 21 and 28 days. The organs namely liver, kidney and pancreas were examined for any gross abnormalities and preserved in 10% formalin, processed by routine paraffin embedding method and stained by haematoxylin and eosin for histopathological examination.

Statistical analysis: The mean and standard error for all the groups was calculated. The mean values were compared with using students' t-test at 5% level of significance.

Results and Discussion

The yield of volatile oil obtained from the fresh leaves of T. capitatus was found to be 2.49%. The present findings were in accordance with that of Alonso (4) who reported a yield of 0.8-2.5% of volatile oil extracted from the fresh leaves and twigs of T. capitatus.

The biochemical findings recorded in both treated as well as untreated rats are shown in Table 1 and 2 and Fig. 1a-d. The untreated diabetic control rats showed significant (p<0.05) progressively increased values of serum glucose, cholesterol, LDL, triglycerides, ALT, AST, serum urea nitrogen and creatinine and decreased values of serum HDL towards the end of the trial. The biochemical findings correlated well with that of histological findings which revealed progressive necrosis of the islet cells of the pancreas (Fig. 1) and degenerative changes in the renal tubules and hepatocytes.

The diabetic rats treated with volatile oil of thyme recorded significant (p<0.05) decreasing values of serum glucose towards normal at the end of the trial, showing a mean value of 483.32±1.35 mg% and 113.20±1.00 mg% on day 0 and day 28, respectively. The serum cholesterol levels showed progressively inclining values from 82.63±0.93 mg% at 0 day to 144.00±1.83 mg% at 14 days and thereafter declining to 107.40±1.20 mg% at the end of the trial. However, serum HDL and triglycerides remained within normal limits throughout the entire period of study while LDL levels started increasing until day 14 (30.70±1.12 and 89.40±0.79 mg% at 0 and 14 days, respectively) and

<table>
<thead>
<tr>
<th>Table 1: Serum glucose and triglyceride levels* in normal, diabetic and thyme oil treated rats</th>
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<tr>
<td>Glucose (day)</td>
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<tr>
<td>Group</td>
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<tr>
<td>--------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
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<td>5</td>
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<td>6</td>
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*: Values expressed in mg% **: Values with different superscripts differ significantly

Effect of Volatile Oil of Thymus capitatus in Alloxan-Induced Diabetic Rats
Table 2: Cholesterol, HDL and LDL levels* in normal, diabetic and thyme oil treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (day)</th>
<th>HDL (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>84.40±0.57</td>
<td>84.64±0.72</td>
</tr>
<tr>
<td>2</td>
<td>84.20±0.65</td>
<td>84.00±0.67</td>
</tr>
<tr>
<td>3</td>
<td>81.43±0.46</td>
<td>83.36±0.77</td>
</tr>
<tr>
<td>4</td>
<td>82.63±0.93</td>
<td>85.52±0.55</td>
</tr>
<tr>
<td>5</td>
<td>84.47±0.62</td>
<td>84.72±0.35</td>
</tr>
<tr>
<td>6</td>
<td>83.07±0.91</td>
<td>84.80±1.02</td>
</tr>
</tbody>
</table>

*: Values expressed in gm% **: Values with different superscripts differ significantly

thereafter started declining, reaching 58.46±0.94 mg% at the end of the trial. The hypoglycemic findings correlated with histological findings which revealed regenerative changes in the islet cells of the pancreas (Fig. 2) towards the end of the trial.

Fig. 1: Showing pancreatic islet cell necrosis-untreated diabetic rat (7 days)

A mild increase in serum ALT and AST in diabetic rats treated with volatile oil of thyme was recorded on day 7 (44.00±1.67 and 42.40±1.46 U L\(^{-1}\), respectively). These enzymes started declining thereafter until the end of the trial to a value of 37.00±1.00 and 21.00±1.00 IU L\(^{-1}\) for ALT and AST, respectively. However, serum urea nitrogen and creatinine levels remained within normal limits until the end of the trial. These biochemical findings correlated with the histological findings which revealed no abnormal changes in the liver and kidney throughout the entire period of the study except for mild degenerative changes in the liver recorded on day 7.

Fig. 2: Showing pancreatic islet cell regeneration-Thyme treated diabetic rat (21 days)

The present hyperglycemic findings observed in diabetic rats might be attributed to cause of destruction of beta cells of the pancreas by alloxan\(^{[34]}\) as confirmed histologically. Abnormal lipid findings could be due to altered

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lipid metabolism consequent to insulin deficiency. In diabetes mellitus, the utilization of impaired carbohydrate leads to accelerated lipolysis resulting in dyslipidemia as a result of insulin deficiency which fails to activate the enzyme lipoprotein lipase and hydrolyse the triglycerides (18). Increased levels of serum ALT and AST levels and decreased values of total protein and albumin might be as a result of hepatic damage (16,18,21). Increased serum urea nitrogen and creatinine concentrations might be due to renal insufficiency that is commonly encountered in uncontrolled diabetes mellitus (12).

The present hypoglycemic findings recorded in thyme-treated diabetic rats were also observed in observed in diabetic rabbits treated with volatile oil of Nigella sativa seeds after 4 and 6 h of treatment and Myrtus communis @ 50 mg kg⁻¹ body weight once daily for a period of 1 week (1,28,29). Similar observations were also recorded in diabetic rats treated with black caraway (Carum carvi L.) oil for a period of 10 weeks (14).

The present antilipidemic findings observed in thyme-treated diabetic rats was also reported in healthy individuals and patients with coronary artery disease treated with garlic oil (11) and in normal healthy humans who received garlic essential oil @ 18 mg day⁻¹ for 4 weeks (10). Similar observations were also observed in rats treated with Nigella sativa oil @ 800 mg kg⁻¹ body weight for 4 weeks and in hypercholesterolemic patients @ 2.5 mL twice daily for 4 weeks (5,13).

The antihyperglycemic activity of thyme might be due to the presence of active principles similar to that of oral hypoglycemic agents which might act by stimulation of the beta cells of the pancreas to release insulin as evidenced histologically. In vitro and in vivo studies revealed that rosmarinic acid and luteolin inhibit the activities of enzymes, alpha glucosidase and alpha amylase and thus preventing the absorption of glucose in the small intestine (17,19,23) (Kim et al., 2000). The presence of such active compounds in T. capitatus might be responsible for the antihyperglycemic activity.

The antilipidemic response of T. capitatus might be due to the restoration of normal lipid metabolism consequent to the antihyperglycemic mechanism. Taku et al. (30) opined that thymol and carvacol significantly decrease serum cholesterol levels by increasing the microsomal geranyl pyrophosphate pyrophosphatase activity. The structural diversity of the isopropanoids which suppress cholesterol synthesis may be reconciled by their ability to increase pyrophosphatase activity, thus leading to the production of the endogenous, post-transcriptional regulator of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Thymoquinone, a derivative of thymol has been reported widely to possess antilipidemic property (2,7). The presence of such compounds in T. capitatus might contribute to their antilipidemic property.

The normal biochemical and histological findings of the liver and kidney in thyme-treated rats might be as a result of restoration of normal functions secondary to antihyperglycemic effects of active compounds present in thyme.

Conclusion

The present study revealed the antihyperglycemic and antilipidemic effects of volatile oil of T. capitatus. However, the exact mechanism and the active compounds involved in hypoglycemic as well as hypolipidemic activities remains to be elucidated. In addition, the present findings also revealed the non-toxic effects of thyme oil when administered @ 0.1mL kg⁻¹ body weight for 28 days as evidenced biochemically and histologically.

References


